A genetic study of the role of chromium in *S. pombe*

Aaron J. Sutherland and Amanda J. Bird

Department of Molecular Genetics, The Ohio State University, Columbus, OH

ABSTRACT

Chromium (III) is a trace nutrient that is considered essential for human life. However, its biological roles in the body are largely unknown. A few studies indicate that Cr^{3+} may enhance the effects of insulin and help the body maintain healthy glucose levels, but to date, clinical trials testing its effectiveness have mostly been inconclusive. As yeast is known to contain particularly high amounts of chromium, the purpose of this study was to use the fission yeast *Schizosaccharomyces pombe* as a model system to gain a better understanding of the mechanisms by which chromium is imported and stored. We have found that *S. pombe* survives on plates supplemented with Cr^{3+} up to a concentration of approximately 3 mM. We have also found that *S. pombe* accumulates Cr^{3+} from its environment, much like brewer's yeast, *Saccharomyces cerevisiae*. To identify genes involved in chromium uptake, we have set up a genetic screen to isolate mutants which are able to survive in the presence of 3.5 mM Cr^{3+}. Our ongoing studies are characterizing a number of these mutants, investigating why they have increased chromium tolerance, and determining whether genes involved in chromium accumulation are conserved between yeast and humans.

BACKGROUND

In the late 1970s, studies were performed on patients receiving long-term total parenteral nutrition who developed diabetes-like symptoms (glucose intolerance, weight loss, neuropathy, etc.). When chromium was added to the patients' feeding solution (150-200 mg/day), their symptoms were reversed. This suggests that the patients suffered chromium deficiency. As a result, chromium is considered an essential nutrient for regulation of normal glucose levels.

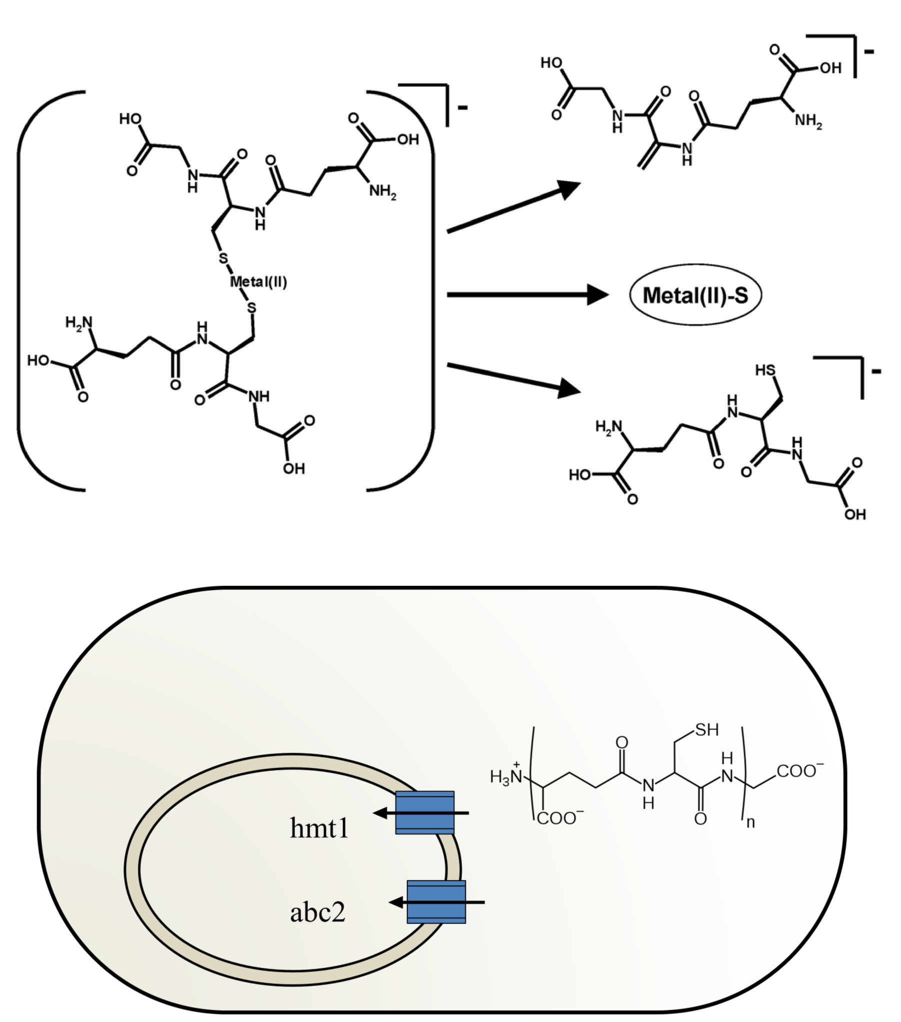

Figure 1. Phytochelatin import in *S. pombe.* Figure adapted from Rubino (2015).

Brewer's yeast, *S. cerevisiae*, is known to be a good source of chromium. For this reason, we speculated that fission yeast, *S. pombe*, would also be rich in chromium and thus an effective model organism for measuring chromium uptake.

A study in 2013 suggested that glutathione or a glutathione-like molecule may bind chromium. As *S. pombe* synthesizes phytochelatins - a glutathione derivative - we hypothesized that phytochelatins may be involved in chromium uptake and accumulation. For this reason, we first focused on known genes related to phytochelatins. Among these were hmt1 and abc2, which transport phytochelatins into vacuoles.

METHODS

To generate strains of chromium resistant mutants, *S. pombe* cells were grown on yeast extract (YE) plates supplemented with chromium (III) chloride ($CrCl_3$). To allow for selective growth, we first determined a concentration which was toxic to wild type cells but at which mutant cells survive. By examining growth in the presence of 0.1 – 5 mM $CrCl_3$, we found the ideal selective concentration to be about 3 mM.

To measure uptake of chromium, we utilized atomic absorption spectroscopy (AAS). Cells were prepared using the following procedure:

1. Growth overnight in 5 ml YES liquid medium
2. Cells were scaled up to 40 ml in YES + $CrCl_3$ and grown for 4 hours
3. Cells were then centrifuged and washed twice with 10 mM EDTA
4. Cells were then boiled in 150 µl concentrated nitric acid for 45 minutes
5. All samples were diluted to 1 ml with 18 MΩ distilled water
6. Total chromium was then measured using AAS

RESULTS

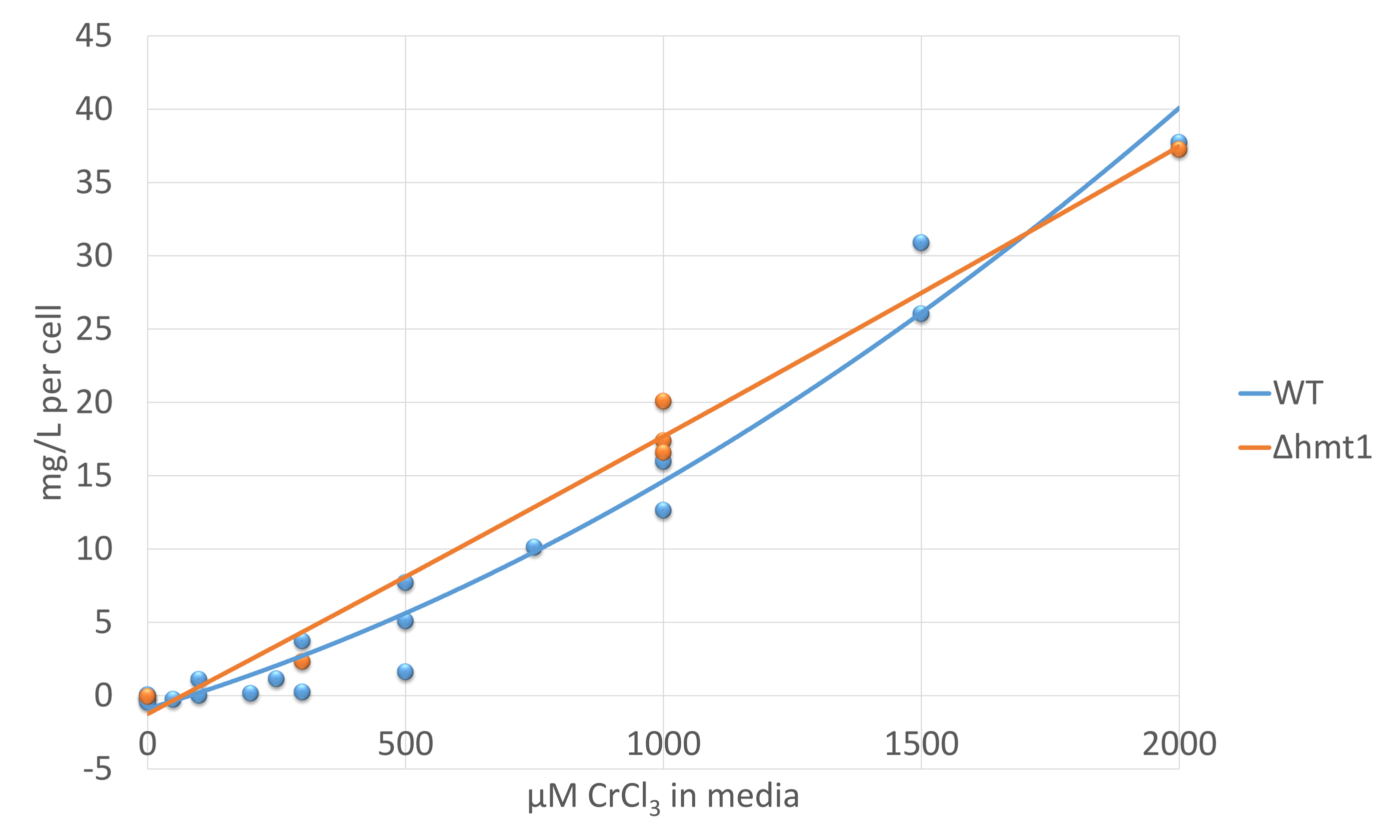

Figure 2. *S. pombe* cells accumulate chromium

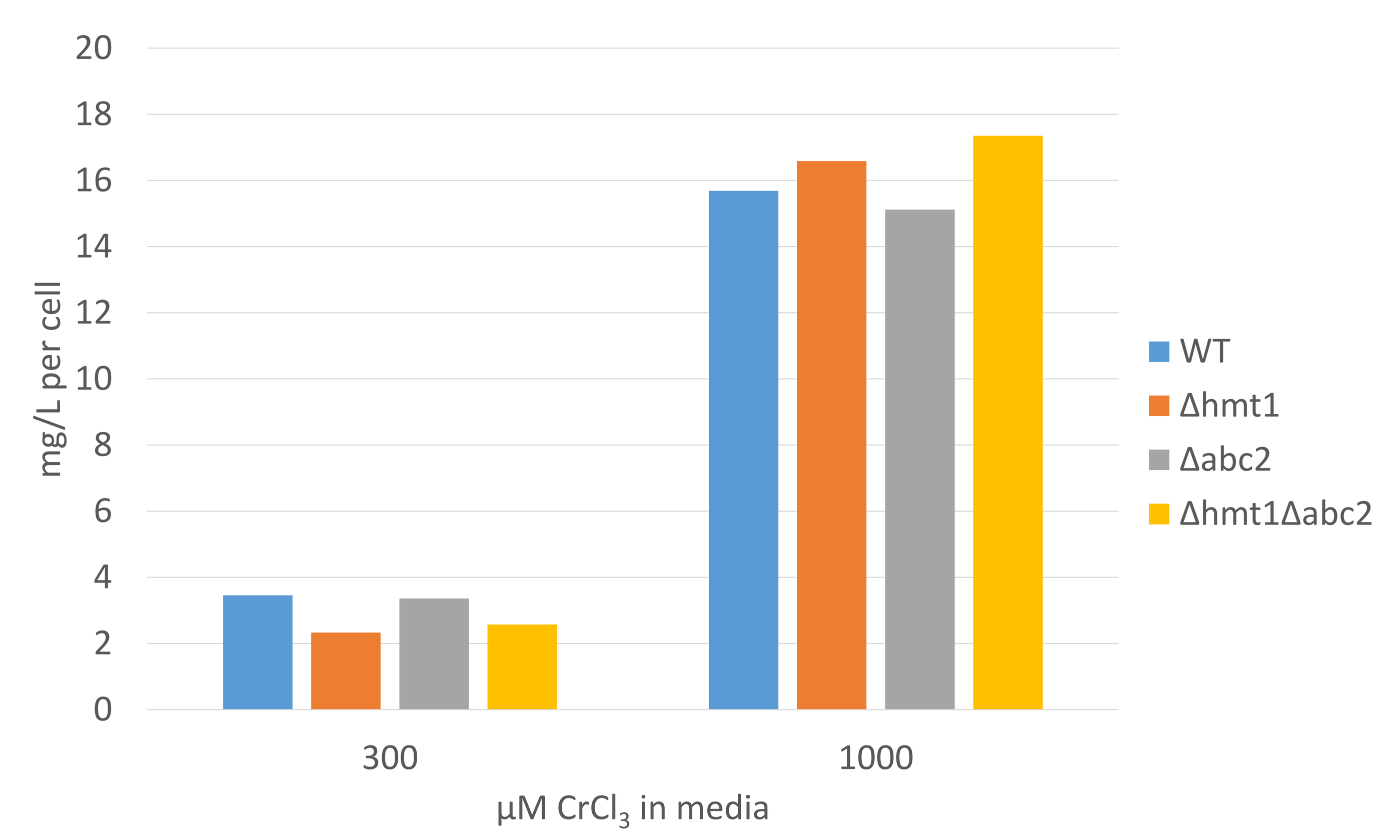

Figure 3. Chromium accumulation in phytochelatin transporter mutants

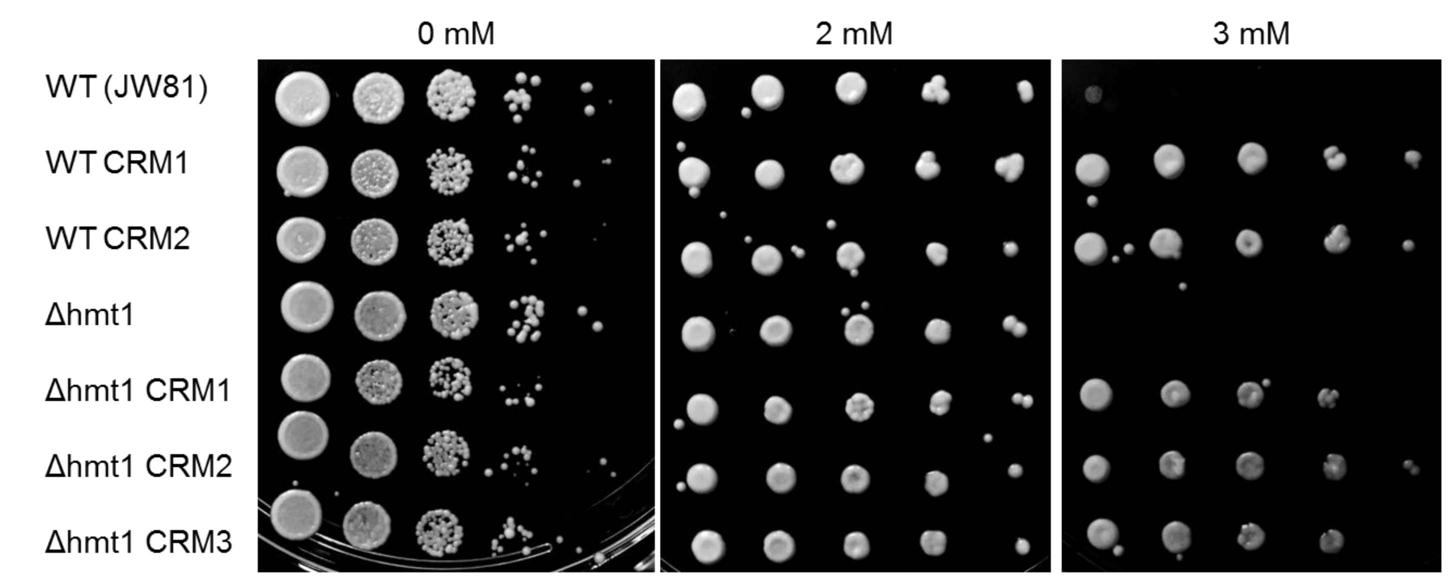

Figure 4. Mutants isolated from screen survive in high chromium media

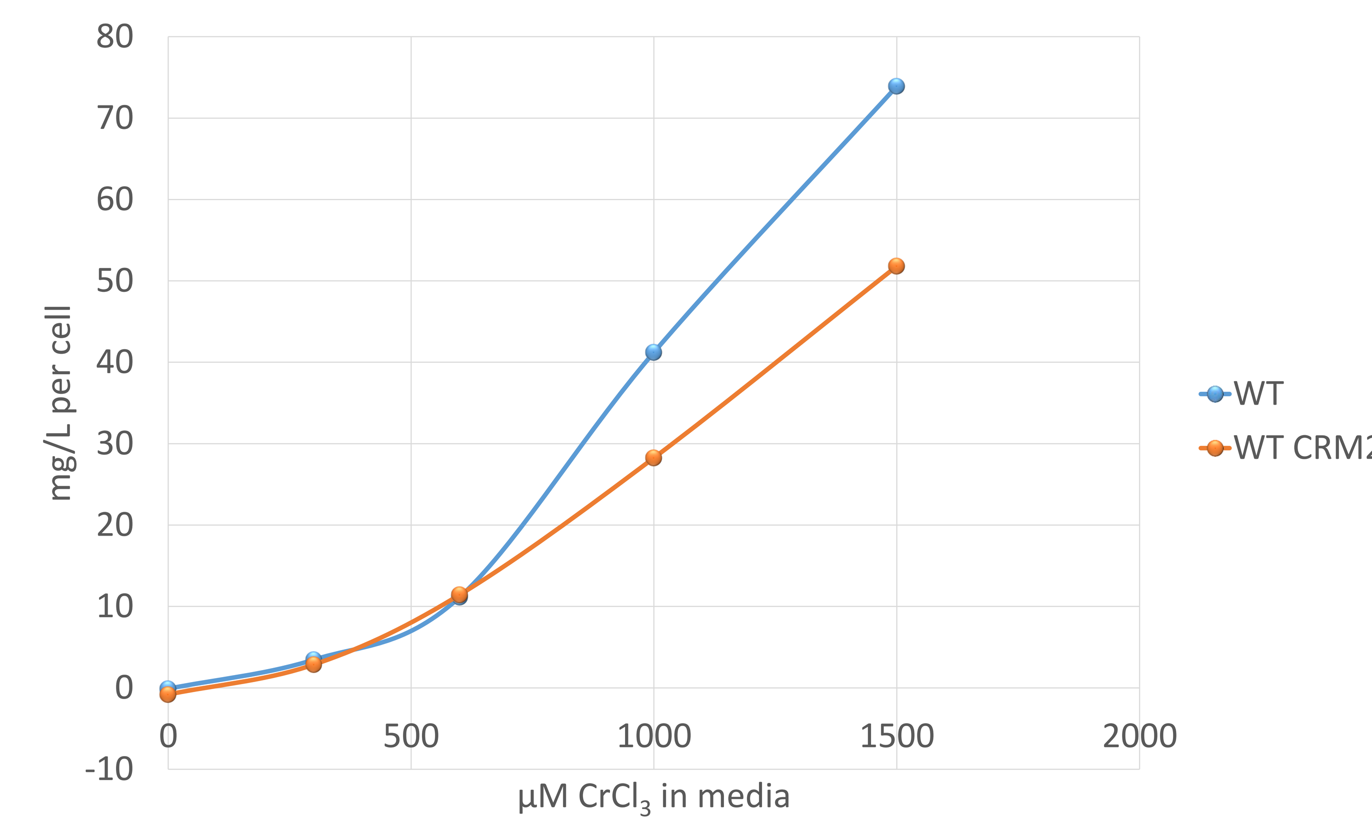

Figure 5. Mutant strain WT CRM2 has reduced chromium uptake

CONCLUSIONS/FUTURE WORK

- No connection between phytochelatin transporters and chromium uptake
- Isolated eight strains of *S. pombe* containing mutations that confer chromium resistance
- At least one mutant has decreased chromium uptake

Our current focus is to perform tetrad analysis on our mutant strains to determine linkage. Another goal is to continue using AAS to determine the changes in chromium uptake for each mutant strain.

REFERENCES

1. Brown, RO, Forloines-Lynn, S, Cross, RE, Heizer, WD. Chromium deficiency after long-term total parenteral nutrition. *Digest Dis Sci* 1986;31(6):661–664.
2. Rubino, F. Toxicity of glutathione-binding metals: a review of targets and mechanisms. *Toxics* 2015;3(1):20-62.
3. Liu, L, Lv, J-P, Uluko, H. Purification and characterization of chromium-binding substances from high-chromium yeast. *J Agric Food Chem* 2013;61(6):1279–1287.